

**SCIENTIFIC CRITERIA
DOCUMENT FOR STANDARD
DEVELOPMENT No. 1-84**

**MICROORGANISMS IN
RECREATIONAL WATERS**

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Ontario

Ministry
of the
Environment

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DOCUMENT FOR STANDARD
DEVELOPMENT No. 1-84**

**MICRO ORGANISMS IN
RECREATIONAL WATERS**

Prepared for

Hazardous Contaminants and Standards Branch

Ontario Ministry of the Environment

by

Expert Committee

May 1984

AMWY

PREFACE

The Hazardous Contaminants and Standards Branch has been assigned the specific mandate of co-ordinating the development of standards (guidelines) for the regulation of various hazardous substances in the environment. These standards must address both human health concerns and the protection of the environment.

A priority list of candidate substances for standard setting was developed by the Ministry of Environment. This list includes microorganisms because of the potential health hazard posed by excessive levels of these organisms in the environment.

In June of 1983, a Standards Co-ordinator was appointed to:

- a) review existing microbiological guidelines for recreational waters, and,
- b) develop the scientific criteria for new standards which would be more closely allied to public health considerations.

Both Internal and External Expert Committees were formed to provide the Co-ordinator with technical direction and a forum for critical review of the document.

This document provides the scientific basis for the formulation of Provincial microbiological standards, following input of public opinion and legal, policy and economic considerations.

This document was prepared under the technical direction of two expert committees.

The committees include the following members:

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Mr. C. A. Burger,	Ministry of the Environment
Mr. G. S. Irwin,	Ministry of the Environment
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Ms. C. Lucyk,	Ministry of the Environment
Mr. G. Palmateer,	Ministry of the Environment
Mr. D. Porter,	Ministry of Health
Ms. A. Vajdic,	Ministry of the Environment
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* Chairman for External Committee review.

EXECUTIVE SUMMARY

In June 1983, the Ontario Ministry of the Environment initiated a review of the Microbiological Water Quality Objectives for recreational waters. The purpose of this review was to develop new standards (guidelines) which would be more closely allied to public health considerations. The review was carried out under the technical direction of an Internal and an External Expert Committee.

The present document presents the scientific criteria for the development of new standards and includes the following recommendations:

1. The use of guidelines based on fecal coliforms should be discontinued because of the lack of correlation between environmental levels of this indicator and human health effects.
2. New microbiological standards based on the indicator organism *Escherichia coli* and the pathogen, *Pseudomonas aeruginosa* should be adopted. The document discusses the relationship between swimming-related illness and levels of the organisms in recreational waters. *Escherichia coli* levels in recreational waters will provide medical authorities with a measure of the potential risk of gastroenteric disease for bathers. *Pseudomonas aeruginosa* will provide a measure of protection for swimmers from *otitis externa* (swimmer's ear).
3. Microbiological standards are effective measures of public health protection if used in conjunction with other beach management criteria, namely:
 - a) sanitary surveys,
 - b) physical and aesthetic water quality considerations,
 - c) constant review of epidemiological information to assess the potential for epidemics.
4. Regular review of these standards is advocated to ensure that they reflect the most recent advances in scientific knowledge.

EXECUTIVE SUMMARY (Cont'd)

The report also recommends standardized field sampling and laboratory protocols for use in conjunction with the new standards. Specifically, sampling at a water depth of 1 to 1.5 m during peak swimming activity and a minimal sampling frequency of 12 samples per sampling site over the swimming season are suggested.

A quality control model which allows health agencies to interpret microbiological data both on the basis of a seasonal geometric mean for *Escherichia coli* and on the basis of more immediate short-term microbiological data, is described in detail.

In addition, the report provides a historical and technical perspective on:

- a) the development and use of microbiological guidelines for recreational waters,
- b) the relevance of microorganisms to human health, and,
- c) the microorganisms of concern in recreational waters.

Research needs, which should be addressed to substantiate the relationships between levels of indicators and pathogens in recreational waters and human health concerns, are presented for management consideration.

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1.0 INTRODUCTION

Ontario's lakes and rivers are the major focal points for many leisure time activities. When these waters are contaminated with human or animal wastes, their use for body contact recreation (swimming, water skiing, etc.) may pose a health hazard for the users.

Pathogenic organisms, such as bacteria, viruses and protozoans enter receiving waters from sewage effluents, storm water outfalls, non-point source runoff and from the bathers themselves. Many of these etiological agents of waterborne disease can survive for long periods in the aquatic environment. Thus, a measure of the suitability of the water for body contact recreation is crucial for the protection of public health. This need for water quality guidelines is especially important in densely populated urban areas where available waterways are intensively used for both recreational purposes and the disposal of industrial and municipal wastes.

Present day microbiological guidelines for recreational waters are typically based on indicator organisms, rather than direct enumeration of pathogens. These guidelines provide a relative measure of the extent of fecal contamination and associated pathogens but are not well based on human health effects.

It is only in recent years that prospective epidemiological studies have provided a data base relating illness, as measured by symptomatology, to several microbiological indicators of water quality. This information, coupled with cost-benefit data, provides a basis for making responsible decisions on acceptable levels of risk to human health versus the loss of recreational facilities.

In light of these developments, the Ontario Ministry of the Environment initiated a review of our existing microbiological guidelines for the purpose of developing new standards that are closely allied to public health considerations. This review was carried out under the technical direction of experts from the Ontario Ministries of Environment and Health, National Health and Welfare, U.S.A. Environmental Protection Agency and the Universities of Toronto and British Columbia.

2.0 HISTORICAL OVERVIEW OF THE DEVELOPMENT OF MICROBIOLOGICAL GUIDELINES FOR RECREATIONAL WATERS

Early efforts in the development of microbiological standards for bathing water commenced with the formation of the American Public Health Association Joint Committee on Bathing Places (A.J.P.H., 1922). In 1924, the Committee proposed a tentative bacterial standard for swimming pools, but no standard was proposed for natural bathing beaches (A.J.P.H., 1924). In 1936, the Joint Committee proposed a classification scheme for natural waters based on coliform counts and suggested that waters with coliform densities up to 1000 per 100 mL could be regarded as acceptable (A.J.P.H., 1935; 1936). Subsequent committee reports in 1939 and 1955 emphasized the paucity of information on the role of natural bathing places in the spread of disease (A.P.H.A., 1957).

The 1000 total coliform (TC) per 100 mL was generally adopted as a guideline by most jurisdictions. In 1951, Streeter attempted a more analytical approach for Ohio waters. He introduced a bather risk factor based on coliform-*Salmonella* ratios, and concluded that waters with 1000 TC per 100 mL would not present a hazard to bathers.

In 1963, at the request of the American Society of Civil Engineers, Sanitary Engineering Division, the Public Health Activities Committee reviewed the status of coliform standards for recreational waters. The Committee found that most U.S.A. states and Canadian provinces recommended the use of voluntary guidelines based on total coliforms. Their report concluded that, "the coliform organisms are used widely in the sanitary classification of bathing waters but there is practically no evidence that would support any of the current numerical values" (ASCE Journal of the Sanitary Engineering Division, 1963).

In 1968, the National Technical Advisory Committee (NTAC) to the Federal Water Pollution Control Administration recommended that fecal coliforms (FC), a sub-group of total coliforms, be used as the bacterial indicator of water quality since they are more specifically related to sewage contamination. They recommended an upper limit of 200 FC per 100 mL for recreational waters.

The NTAC recommendations were based on epidemiological studies conducted by the U.S. Public Health Service in the late 1940's and early 1950's and summarized by Stevenson in 1953. These studies were conducted at two freshwater sites on Lake Michigan and the Ohio River and two marine sites. Swimming-associated gastroenteritis was not observed at the marine sites nor at the Lake Michigan sites. However, the Ohio River study showed increased gastrointestinal illness at median coliform densities of about 2300 per 100 mL. Data subsequently collected from the Ohio River site during the 1960's suggested that a level of 400 FC was approximately equivalent to the threshold number of 2300 total coliforms (Geldreich, 1966). Following application of a safety factor, the 200 FC per 100 mL guideline was developed. Although the design of Stevenson's studies has been severely criticized (Moore, 1975; Cabelli *et al*, 1975), it is apparent that these studies have formed the basis of most guidelines in use today.

The NTAC guideline of 200 FC per 100 mL is now the most widely used criteria for recreational waters in North America. It was adopted by the U.S.A. Environmental Protection Agency in 1972 and by the Canadian Government (National Health and Welfare) in 1983, although some agencies have adopted more stringent guidelines (Table 1).

Extensive sampling of Ontario beaches during the 1960's revealed that FC levels were generally well below 100 per 100 mL unless a significant pollution source were present. Ontario's guideline of 100 FC per 100 mL was first recommended in 1970 as a means of protecting the quality of our recreational waters and providing a greater degree of protection to the public.

TABLE 1. RECREATIONAL WATER GUIDELINES IN SELECTED JURISDICTIONS

<u>Jurisdiction</u>	<u>Total Coliforms</u>	<u>Fecal Coliforms</u>
CANADA		
Federal	-	200
British Columbia	-	200
Alberta	1000	200
Manitoba	100 (MPN)	20 (MPN)
Ontario	1000	100
Newfoundland	500	20
New Brunswick	-	200
Nova Scotia	2500	200
OTHER		
U.S.-EPA	-	200
W.H.O	-	100
U.S.S.R.	1000	-
Greece	500	-
Italy	-	100
Belgium	-	200
Sweden	-	100

3.0 RELEVANCE OF MICROORGANISMS IN RECREATIONAL WATERS TO HUMAN HEALTH

An adverse health effect has been defined as, "a biological change which reduces the level of well being or functional capacity. These biological changes can range from trivial to fatal", (Higgins, 1983). Most infectious diseases transmitted by recreational waters although not necessarily trivial, do not constitute a serious illness and are not usually reported to or by medical authorities. The most common swimming-associated illness in recent studies in the U.S.A. was a relatively benign gastroenteritis of short duration suspected to be caused by a rotavirus or Norwalk-like virus. The symptoms typically included one or a combination of: vomiting, diarrhea, stomach ache or nausea (Cabelli, 1983a; Dufour, 1984).

Potentially, any infection where the etiological agent is excreted in the feces, can be transmitted to bathers through swimming in sewage contaminated waters (Cabelli, 1983a). These include enteric bacterial diseases such as salmonellosis (including typhoid), shigellosis, cholera, and viral diseases such as infectious hepatitis. Common non-enteric infections include swimmers ear (*otitis externa*), swimmer's itch and various boils and rashes (Cabelli, 1978; Cabelli, 1983a).

There are few instances of well-documented outbreaks of disease associated with recreational waters (Schiemann, 1976; U.S. Department of Health and Human Sciences 1981, 1982). Fewer than 20 outbreaks of enteric disease have been reported in the literature and most of these appear to be associated with swimming in heavily polluted waters (Dufour, 1984). Even in cases of documented outbreaks, monitoring of the water often took place well after illness was apparent, thus making the link to conditions prevalent at the time of swimming rather tenuous.

According to Discher (1963), the first documented report of bathing-associated illness was an outbreak of 49 cases of typhoid in 1888, following swimming in the Elbe River. The American Public Health Association reported a similar typhoid epidemic at a boy's camp in 1921 (A.P.H.A., 1922). Typhoid epidemics in Canada and the U.S.A. between 1920-1936 were documented by Gorman and Wolman (1939). They reported only four outbreaks involving 35 cases that could be attributed to swimming activities.

With the exception of an outbreak of salmonellosis in Australia in 1958 (Anonymous, 1961), diseases attributed to *Salmonella* have declined since the 1940's. It has been suggested that this decline is a result of sanitary advances, including improved sewage treatment and disinfection (Discher, 1963; Dufour, 1984). Most swimming related enteric disturbances now appear to be of viral origin (Dufour, 1984).

Although poliomyelitis has been a disease of major concern, no clear evidence has established recreational waters as a source of transmission. In a study in England, Moore (1959) monitored the swimming experiences of children with diagnosed poliomyelitis and a comparable disease free control group. He concluded that swimming activities were unrelated to the transmission of this disease.

It has been suggested that reports of outbreaks of swimming-associated disease may understate their actual occurrence (Cabelli, 1983a). With regard to various mild forms of gastrointestinal disturbances, the lack of evidence is quite understandable since most people would not seek medical attention. Also, many of the enteric diseases are transmissible by other means, including drinking water, contaminated food and person to person contact. Consequently, the source of the etiological agents is extremely difficult to trace unless an outbreak occurs within a geographically confined area such as a summer camp.

3.1 PATHOGENIC ORGANISMS

From a health perspective, it would appear to be logical to routinely examine recreational waters for the pathogens (etiological agents), such as *Salmonella* and *Shigella*, which can cause waterborne disease. There are, however, a number of reasons why this is not a common practice. Firstly, there are a great variety of infectious organisms which can be transmitted by a waterborne route. Since their densities are not interdependent and can vary both temporally and spatially, separate measurements for each pathogen would be required. Secondly, pathogen densities in feces and sewage and, consequently, in receiving waters, are extremely variable. Their densities are dependent on the number of carriers in the discharging population. Finally, the relationships between the recovery of pathogens from recreational waters, the frequency of transmission and the hazard to public health are unclear.

Perhaps the greatest barrier to the routine monitoring of pathogens is the unavailability of practical and reliable methods for their enumeration. For example, investigations into the occurrence of viruses in recreational waters have been hampered by the limitations of virus concentration and recovery methods. Current methods are relatively expensive, time consuming, require highly trained personnel and are only capable of recovering a narrow spectrum of the enteric viruses which may be present in the water.

One of the notable exceptions is *Pseudomonas aeruginosa* for which suitable enumerative methods have been developed (Levin and Cabelli, 1972). This pathogen has been identified as the major etiological agent for "swimmers ear" (*otitis externa*) (Cabelli, et al, 1974). Research studies have shown a relationship between the numbers of *P. aeruginosa* in the water and the incidence of ear infection. Therefore, *P. aeruginosa* has been proposed as a measure of the risk of ear infections in the bather population.

In summary, tests for most pathogenic organisms are recommended mainly as supplementary measures for special investigations of suspected waterborne diseases or where an outbreak of disease among a potential user population has occurred. Some of the more important causative agents of waterborne disease are described in more detail in Appendix I.

3.2 INDICATOR ORGANISMS

Environmental microbiology presents some unique problems. Unlike clinical situations, pathogenic organisms in the aquatic environment are usually present in low numbers, irregularly distributed and, thus, difficult to isolate and quantify. Consequently, it is common practice to monitor surrogate groups of organisms (indicators) as a measure of water quality. These indicator organisms are far more abundant in the environment than pathogens and are, therefore, easier to detect.

Historically, the main concern with waterborne pathogens has been the potential spread of infectious enteric diseases, such as cholera and typhoid, whose etiological agents are usually present in waters contaminated with fecal wastes. Thus, it is not surprising that the water quality indicators used in environmental microbiology are groups of organisms that are present in the feces of all warm-blooded animals in numbers far exceeding pathogens. The presence of elevated levels of the indicators in the environment is indicative of fecal contamination and associated hazard from pathogens.

The desirable characteristics of an ideal fecal indicator have been summarized by the National Academy of Sciences (1977) as follows:

- (a) suitable for use in all types of water;
- (b) present in sewage and polluted waters when pathogens are present;
- (c) their number is correlated to the amount of pollution;
- (d) present in greater numbers than pathogens;
- (e) do not proliferate in the aquatic environment;
- (f) survive for longer periods in the environment than pathogens;
- (g) absent from unpolluted waters;
- (h) readily detected by simple and accurate laboratory methods, and;
- (i) harmless to man and animals.

At present, the most widely used indicators of recreational water quality are the total and fecal coliforms. These indicators are, however, defined by methodological rather than precise taxonomic criteria and, thus, include a variety of organisms rather than just the definitive fecal bacteria.

The total coliform test enumerates bacteria in the family, Enterobacteriaceae: *Escherichia*, *Klebsiella*, *Citrobacter* and *Enterobacter* and may also include *Aeromonas*. The use of this indicator has been discontinued by many jurisdictions since it is now recognized that some of the genera within this group (e.g. *Klebsiella*, *Citrobacter* and *Enterobacter*) are not fecal specific. In fact, total coliforms can be isolated from most waters, including those essentially untouched by man (Goodrick et al 1970). Sources of coliforms in water uncontaminated by human or animal wastes include soil, air and natural proliferation under nutrient rich conditions.

In 1968, the National Technical Advisory Service recommended the use of fecal coliforms, a sub-group of total coliforms since they are more fecal specific. The fecal coliform test enumerates: *E.coli*, *Klebsiella*, *Enterobacter* and *Citrobacter*. Only *E. coli* is considered a definitive fecal coliform. *Klebsiella* is infrequently present in human feces and will furthermore proliferate in carbohydrate enriched aquatic environments. Thus the fecal coliform group suffers from some of the same shortcomings as the total coliform indicator (Cabelli, 1983a).

In 1983, Cabelli *et al* noted that "ideally, recreational water quality indicators would be microorganisms whose densities in the water could be quantitatively related to swimming-associated health hazards". It is only in recent years that research studies have attempted to define the exposure-response relationship between several microbiological indicators of water quality and illness in the bather population (Cabelli, 1983a; and Dufour, 1984). The bacterial indicators used as a measure of water quality in these studies included *E. coli*, *Enterococcus* and fecal coliforms. *Good correlations were obtained between the incidence of swimming-associated illness and both E. coli and enterococcus densities in the water. No such relationship was found for the fecal coliform indicator.*

Table 2 compares the characteristics of these three indicators. It is apparent that both *enterococcus* and *E. coli* more closely fulfill the requirements of an "ideal indicator" than do the more heterogeneous fecal coliform group. In fact, the advantages of *E. coli* were recognized by the Canadian Federal Government in their recently published Guidelines for Canadian Recreational Water Quality (1983). This document states: "If the methodology for enumeration of *E. coli* were as simple, easy and well standardized as methods for fecal and total coliforms, it would be the indicator of choice for recreational waters."

Although highly significant relationships between the density of enterococci and incidence of gastrointestinal disease in bathers have been developed for both marine and freshwater beaches in the U.S.A. (Cabelli 1983, Dufour 1984), *the development of an Ontario standard for Enterococcus is currently hampered by the paucity of Canadian data on its occurrence and by lack of agreement on acceptable laboratory protocols.* Pending resolution of these difficulties, *enterococcus* may be adopted as a comparative or alternative indicator to *E. coli*.

TABLE 2. IDEAL INDICATOR CHARACTERISTICS IN THREE INDICATORS OF FECAL CONTAMINATION (modified from Dufour, 1983)

<u>Ideal Characteristics</u>	<u>Enterococci</u>	<u>E. coli</u>	<u>Fecal Coliforms</u>
1. Present when pathogens are	Yes	Yes	Yes
2. Unable to grow in aquatic environments	Yes	Yes	No
3. More resistant to disinfection than pathogens	Yes	No	No
4. Easy to isolate and enumerate	Yes	Yes	Yes
5. Applicable to all types of water	Yes	No	No
6. Exclusively associated with fecal wastes	Yes	Yes	No
7. Occur in greater numbers than pathogens	Yes	Yes	Yes
8. Density of indicator should have direct relationship to degree of fecal contamination	Yes	Yes	No
9. Indicator density should correlate with health hazard from a given type of pollution	Yes	Yes	No

Total staphylococci have also received considerable attention as a potential indicator for recreational waters. They have been specifically proposed as a measure of the impact of bather density on water quality. *Studies on the relationships between the densities of total staphylococci and bather illness are currently in progress and any decision regarding its practical use as a standard must await completion of these ongoing efforts.*

4.0

DERIVATION OF THE MICROBIOLOGICAL STANDARDS FOR
RECREATIONAL WATERS

The Internal and External Expert Committees of the Microbiological Standard Setting program have recommended the use of E. coli and P. aeruginosa as recreational water quality indicators. A substantial data base on typical levels of these organisms at Ontario beaches is now available and practical laboratory protocols have been developed. E. coli levels in recreational waters will provide medical authorities with a measure of the potential risk of gastrointestinal disease for bathers and the recommendations for P. aeruginosa should provide a measure of protection for swimmers from otitis externa. Similar standards have been recently proposed by the International Joint Commission (IJC, 1983).

To ensure that the Provincial standards reflect the most recent scientific advances in public health protection, regular review is strongly recommended.

It is also important to emphasize that any microbiological standards are only an effective measure of public health protection if used in conjunction with other beach management criteria,* namely:

- (a) Sanitary surveys;
- (b) Physical and aesthetic water quality considerations;
- (c) Constant review of epidemiological information to assess the potential for epidemics;

ESCHERICHIA COLI

4.1

4.1.1. Description

E. coli are Gram-negative, facultatively anaerobic, non-sporeforming rod-shaped bacteria that occur singly or in from other members of the family Enterobacteriaceae by a series of biochemical tests (IMViC reaction). *E. coli* typically produce indole, and give a positive methyl red test. *E. coli* do not produce acetyl methyl carbinol and cannot utilize citrate as a sole source of carbon.

* These criteria are currently under review by a separate Ministry of Health, Public Health Branch, Committee.

Because, in the past, it was necessary to use a fairly long and relatively expensive testing procedure to separate *E. coli* from other enteric bacteria of the same family, the total and fecal coliform tests were established as indicators of the presence of fecal contamination. It was, however, always recognized that *E. coli* were the organisms of choice for the detection of sewage contamination. Recently, Dufour (1975 and 1981) devised a more simple membrane filtration method (m-TEC method) that is quite specific for *E. coli*, (Pagel *et al* 1982). The *E. coli* target organism is defined as that typical colony grown on the m-TEC-Ig media described under the laboratory methods section.

4.1.2. Occurrence and Source:

E. coli are inhabitants of the gastrointestinal tracts of man and other warm-blooded animals. Dufour (1977) found that *E. coli* make up 90-100% of all coliforms in the feces of domestic animals. They are rarely, if ever, found growing in natural environments. Therefore, *E. coli* can be used as specific, reliable indicators of fecal contamination of water.

E. coli concentrations vary widely in aquatic habitats in Ontario. Their numbers range from less than 1 *E. coli* per 100 mL in some recreational lakes, to thousands per 100 mL in areas contaminated with sewage or stormwater. Based on available data, it appears that the percentage of *E. coli* in the traditional fecal coliform test is also quite variable, ranging from 63 to 100% in samples from recreational waters (Table 3). The relationship appears to be so variable that it would be difficult to apply a general average. Thermotolerant *Klebsiella* and *Citrobacter* make up a portion of the fecal coliform number, and they have been known to survive, and indeed multiply, in an organically rich environment. *Klebsiella*, for example, thrive in pulp and paper mill effluents (Bell *et al* 1978 and Rokosh *et al* 1977) and in textile industry effluents (Vlassoff 1977). The great fluctuations in the percentage of *E. coli* as part of the fecal coliforms are probably largely due to the *Klebsiella* component of the fecal coliform group.

**TABLE 3. MINISTRY OF THE ENVIRONMENT AND MINISTRY OF HEALTH
MICROBIOLOGICAL DATA ILLUSTRATING THE *E. COLI*
FRACTION OF THE FECAL COLIFORM LEVELS IN TYPICAL SAMPLES.**

ONTARIO SOURCES	<i>E. COLI</i> AS A PERCENTAGE OF FECAL COLIFORM LEVELS
Toronto Waterfront	63 to 95% (GM)
Several Lake Huron Beaches	97 to 100%
Several beaches on smaller inland lakes	90% (overall GM)
Ottawa beaches	90 to 100%
Pulp and paper mill effluent	1% (overall GM)
Sewage effluent (may contain industrial effluents)	54 to 100%

4.1.3. Relationship to Human Health

Studies by Dufour (1984), Ktsanes *et al* (1979), and Seyfried *et al* (1983) indicate that the risk of contracting gastrointestinal illness is greatly increased if a person swims in water contaminated with domestic wastes. Recent epidemiological studies have found a strong relationship between levels of *E. coli* in recreational waters and the incidence of swimming-associated gastroenteric symptoms (Cabelli *et al*, 1983; Dufour, 1984).

These epidemiological studies were conducted at several sites in the U.S.A., including freshwater beaches at Keystone Lake, Oklahoma and Erie, Pennsylvania. At each lake, two separate sites were monitored, one with good water quality and one impacted by sewage outfalls. The beach surveys were conducted on weekends to take advantage of the large populations as well as to permit monitoring of the water quality during peak swimming activity. In total, 28,966 swimmers and 9,274 non-swimmers participated in the study over a 3 year period from 1979 to 1982.

Swimming was rigidly defined as complete immersion and "significant exposure of the upper body orifices to the water". The non-swimming control group were selected from beachgoers who did not meet this definition of swimming. Interviewers solicited information from each participant on age, sex, ethnicity, etc., and also whether the person swam and got his head and face wet, length of time and time of day in the water, and the illness symptoms they may have had in the previous week. If an individual had gone swimming in the previous five days, they were not asked to participate in the study. Telephone interviews were conducted 8 to 10 days after the swimming experience. The relationship for the incidence of swimming-associated illness was derived from the differences in symptomatic illness rates between swimmers and non-swimmers and the density of bacterial indicators in the water.

Figure 1 illustrates the significant relationship between seasonal mean concentrations of *E. coli* and the incidences of bather illness. The slope of the regression line was calculated using illness rates for "highly credible gastrointestinal symptoms" (gastroenteritis). Gastroenteritis is defined as any one of the following: (1) vomiting, (2) diarrhea with fever, and (3) stomach ache or nausea accompanied by fever. The correlation coefficient for the association between gastroenteritis and *E. coli* densities was 0.804.

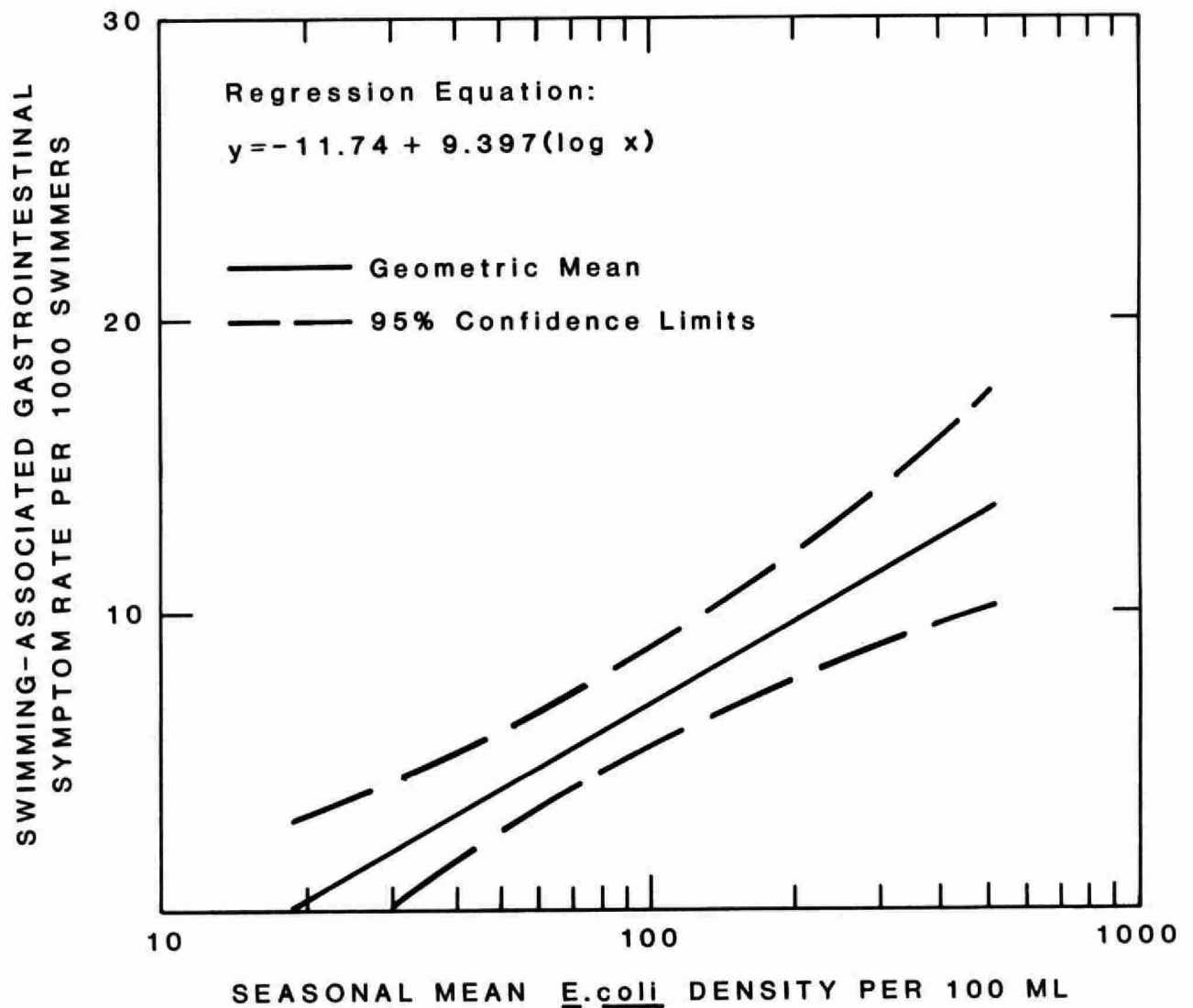


Figure 1: Relationship between swimming-associated gastrointestinal disease and E. coli (seasonal GM) densities in recreational waters, 95% confidence limits (from Dufour, 1984).

Similar epidemiological studies were carried out by Seyfried and Brown (1983) on several inland Ontario beaches, not impacted by industrial wastes. A total of 1,366 swimmers and non-swimmers was interviewed during this study. From these interviews, information was accumulated on bathing activities and incidences of swimming-related illness. The results indicated a statistically significant difference in illness rates between swimmers and non-swimmers. The study also suggests some correlation between fecal coliform densities and bather illness rates ($p = 0.0058$). Although this study used fecal coliform results, typing of the typical fecal coliform colonies indicated that *E. coli* accounted for over 90% of the fecal coliform count, thus supporting the relationship between bather illness rates and *E. coli* levels.

4.1.4. Limitations of the Data:

1. The rationale for the use of guidelines or standards based on the densities of a fecal indicator assumes a relatively constant ratio between the indicator and pathogens in sewage effluents and consequently the receiving waters. This relationship could change under epidemic conditions.
2. The morbidity data collected on swimmers and non-swimmers have been averaged over an entire season. The levels of indicator organisms are compared with this seasonal average of epidemiological data. The microbiological data cannot be used to assess the risk of disease on any specific day. It should also be remembered that the microbiological results are not available until at least the day after the samples are taken, hence the system is not set up for immediate response.
3. The *E. coli* samples for the epidemiological survey were taken in waters 1 to 1.5 meters in depth. This appears to ignore the risk to the toddlers that bathe in the shallow waters. The study, however, addresses that area by analyzing morbidity data on all bathers, no matter the age or where they swam. The objectives should thus protect all bathers including the children bathing in the shallow areas.
4. It has been estimated that only about 30% of the swimmer-associated ailments are gastrointestinal (Dufour, 1984). Hence *E. coli* as an indicator of risk to public health is fairly limiting in terms of overall swimmer-associated morbidity.

4.1.5. Recommended Standard:

E. coli is recommended as the indicator of choice for recreational waters in Ontario. Based on the relationship described in Figure 1, a maximum upper limit for *E. coli* can be established once an acceptable level of risk of gastrointestinal disease for swimmers versus the loss of recreational facilities has been defined. At a mean density of 18 *E. coli* per 100 mL, there is no significant difference in illness between the swimming and non-swimming population. About 7 swimmers per 1000 (or 0.7%) are at risk of contracting gastrointestinal symptoms at mean *E. coli* densities of 99 per 100 mL. At 210 *E. coli* per 100 mL, 10 swimmers per 1000 (1%) may develop gastrointestinal symptoms.

4.2 PSEUDOMONAS AERUGINOSA

4.2.1. Description:

P. aeruginosa is a motile Gram-negative, rod-shaped bacterium which produce oxidase, pyocyanin and fluorescein. This organism typically requires minimal growth factors, and can multiply in mineral media containing very low levels of organic material. *P. aeruginosa* exhibits extensive biochemical versatility and resistance to anti-microbial agents. The *P. aeruginosa* target organism is that organism that gives a typical colony when grown on the m-PA medium as described in the laboratory methods section.

4.2.2. Occurrence and Source:

P. aeruginosa has been considered ubiquitous in U.S. waters, but studies done by the M.O.E. on lakes in Ontario indicate that *P. aeruginosa* is most likely to be found in bathing areas impacted by high human activity. Both sewage and bathers themselves are a possible source of this organism in recreational waters. In raw domestic sewage, concentrations of 10^5 to 10^6 *P. aeruginosa* per 100 mL are common, since slightly in excess of 10% of the healthy adults in the United States are intestinal carriers of *P. aeruginosa*. *Pseudomonas* levels in excess of 100 organisms per 100 mL can be measured in waters receiving surface drainage from urban areas or water recently contaminated with sewage.

Lanyi *et al* (1966) noticed that *P. aeruginosa* survived longer in waters than did coliforms. Drake (1966) suggested that *P. aeruginosa* levels from 1 to 10 per 100 mL could be expected in rivers with low but definite sources of contamination. Levels of *P. aeruginosa* in Ontario recreational waters range from 0 per 100 mL to greater than 100 per 100 mL, and the median level is typically less than 1 per 100 mL (Burger, 1983).

4.2.3. Relationship to Human Health

P. aeruginosa is a bacterium known to cause skin rashes (Kush & Hoadley, 1980) and eye infections (Wilson and Ahearn, 1977) and is the primary organism associated with ear infections (*otitis externa*) (Alcock, 1977; Cassisi *et al*, 1977). A relationship has been developed between levels of *P. aeruginosa* in the bathing waters and the risk of contracting ear infections.

Hoadley and Knight (1975) reported that the frequency of "earaches" among swimmers was 2.4 times higher than among non-swimmers. They also noted that 78% of the infected ears of the swimmers, and 33% of the infected ears of the non-swimmers contained *P. aeruginosa*. This relationship of *Pseudomonas* to ear infections is supported by Burger (1983), Calderone and Mood (1982) and Jones (1965).

If *P. aeruginosa* is present in the water, immersion of the ear allows water to penetrate the ear canal and may lead to either colonization or infection by this organism. The actual process of infection is not understood, and appears to be, in part, related to the sensitivity of the individual to *P. aeruginosa* infections. Once *P. aeruginosa* have either colonized or infected an ear, these organisms can be spread into the water if the individual allows the infected ear to be immersed while swimming (Seyfried and Cook, 1984).

Data from a four year comprehensive study, carried out by the MOE, were used to develop a relationship between the concentration of *P. aeruginosa* in the bathing waters and the risk of an ear infection. The study included sampling of shoreline and midlake stations in both populated and unpopulated lakes, as well as beaches with public access. Physicians and hospital staff were invited to co-operate by providing medical information and by supplying bacterial swab samples from the ears of their *otitis externa* patients. Ear swabs were also collected from volunteer bathers and non-bathers. Swabs were taken from bathers' ears both before and after swimming.

In 1978, 38 people (10%) of the 380 people interviewed, reported having an ear infection during the summer. Seventy-one percent of the people reporting ear infections were 5 to 14 years old, and 95% had previously experienced ear infections. Similarly, in 1979, 79 of the 1,042 people interviewed (8%) reported experiencing an ear infection. Seventy-five percent of those reporting ear infections were 14 years old or younger, and 90% of them had experienced ear infections previously. Most of the people reporting ear infections swam frequently (Table 4).

The study data indicate that *P. aeruginosa* were present in ear swabs from 3% of healthy swimmers prior to swimming and 8% of the bathers' ears following swimming. The study also showed that *P. aeruginosa* levels tended to increase as the number of swimmers increased since the organism was more easily isolated from heavily used beaches than from swimming areas fronting on private cottages.

A total of 1,612 *P. aeruginosa* were serotyped in 1977 and 1978. The frequency of isolation of typable *P. aeruginosa* was much higher at public beaches than in samples from mid-lake stations. The results of the serotyping and the ear swabbing program support the proposal that the major source of *P. aeruginosa* in bathing waters are the swimmers themselves, with water acting as the vector for transmission from one swimmer to another.

Burger (1983), using these data, found that an ear infection rate of 12 per 100 bathers could be related to the 75th percentile of *P. aeruginosa* levels in the range of 10 to 100 per 100 mL. An ear infection rate of less than 8 per 100 bathers, occurred when the 75th percentile levels were 2 or less *P. aeruginosa* per 100 mL. Median *P. aeruginosa* levels were less than 1 per 100 mL in all cases.

4.2.4. Limitations of data

1. *P. aeruginosa* are usually isolated in low numbers from recreational waters. Typically, many of the data are reported in the "less than" category which does not lend itself to traditional statistical interpretation. The development of a relationship between bather illness rates and ambient densities of *P. aeruginosa* was accomplished by using the 75th percentile method.

TABLE 4 **THE FREQUENCY OF SWIMMING ACTIVITY OF THE STUDY POPULATION (1,042 PEOPLE) AND THOSE REPORTING AN EAR INFECTION IN 1979 (MODIFIED FROM BURGER, 1983).**

Frequency of Swimming	RESPONSE	
	Study Population	Those Reporting an Ear Infection in 1979
Did not swim	51	2%
Occasionally to Frequently	406	4%
Very frequently	584	10%

Occasionally to Frequently - 1-4x/week.

Very frequently - 4 or more times/week.

2. The levels of *P. aeruginosa* in a bathing area will be influenced by the bather density especially the presence of *P. aeruginosa* carriers or individuals with *P. aeruginosa* infections.
3. *P. aeruginosa* have only been related to the risk of *Botitis externa*. These organisms have not been statistically related to other bather afflictions.

4.2.5 Recommended Standard

P. aeruginosa is proposed as a standard for protection of swimmers from *otitis externa*. On the basis of available evidence, if the levels of *P. aeruginosa* exceed 10 per 100 ml, in more than 25 percent of the seasonal samples, *otitis externa* symptoms can be expected to occur in 12% of the bather population.

5.0 STANDARD IMPLEMENTATION

The appropriate field sampling, laboratory and data interpretation protocols required for implementation of the new microbiological standards for recreational waters are described in the following sections:

5.1 FIELD METHODS

A recent survey of 43 Ontario health units showed considerable variation in the field sampling methodology used by these agencies in their beach monitoring programs (MOE, MOH, 1983). This variability in approach has, in the past, caused considerable confusion over the interpretation of microbiological data and the subsequent need to placard beaches which exceeded the 100 fecal coliforms per 100 mL objective.

It is evident that only the adoption of a standardized field sampling protocol by all agencies involved can generate comparable data and ensure uniform application of the microbiological standards in all jurisdictions.

The field sampling protocol recommended in the present document reflects the methods used by Dufour (1984) in developing the relationships between the indicator organisms (*E. coli*, enterococcus) and the incidence of bather illness. Of particular significance in this regard is the 1 to 1.5 m sampling depth used in their studies. A similar sampling protocol was used by Burger for enumerating *P. aeruginosa* (Burger, 1983).

Although the use of this sampling depth may not be feasible at all Ontario beaches, it must be recognized that an alternate sampling depth could invalidate the relationship between bather illness and the presence of *E. coli*. Consequently, in these situations, more reliance must be placed on other criteria such as sanitary surveys to establish the need to placard a beach.

5.1.1. Location of Sampling Site

The location and number of sampling sites must be permanently fixed following a detailed sanitary survey. These sites should be representative of the water quality throughout the beach and should include sampling points in the areas of greater bather activity, as well as in areas potentially impacted by drains, storm sewers, river or stream inputs.

5.1.2. Sampling Depth

Microbiological samples must be collected in waters 1 to 1.5 m deep and about 15-30 cm below the water surface. At beaches where very shallow waters preclude sampling at this depth, samples should be collected as far off shore as possible, within the swimming area. Care must be taken to avoid contamination of samples with surface film and sediments.

5.1.3. Frequency of Sampling

The minimum recommended sampling frequency is 12 samples per sampling site over the bathing season. This frequency was derived by methods described in the ASTM manual on quality control (1951) and represents the smallest number of samples which could generate a representative seasonal average. At this level of sampling, the incidence of bather illness may exceed the acceptable illness rate by 300 per 100,000 bathers or 0.3% of the bather population. This minimal level of sampling intensity should only be used for beaches with historically uniform water quality and little seasonal variation. More frequent sampling is essential for beaches that are affected by intermittent pollution sources (e.g., storm sewers, agricultural drains) or are known to have variable water quality. Samples should be collected during peak swimming activity.

5.1.4. Meteorological Factors

The impact of meteorological factors, such as rainfall, wind direction, intensity of sunlight, water temperature on the bacterial quality of water will be addressed by the MOH Committee in conjunction with other beach management criteria. However, it is suggested that meteorological information be collected and recorded at each sampling time so that each health agency can develop an ability to predict the impact of these factors (e.g., rainfall) on the microbiological water quality.

5.1.5. Sample Containers

Samples must be collected in pre-sterilized glass or plastic bottles containing Sodium thiosulphate, issued by the MOH or MOE laboratories (100 mg/L final concentration).

5.1.6. Sample Transport to Laboratory

Samples should arrive at the Laboratory as soon after collection as possible, preferably on the day of collection. During transport, sample temperature should be maintained below 10° C (preferably at or near 4° C) using refrigeration or ice in insulated containers. Sampling should only be done when laboratory analysis can be done within 24 hours.

5.1.7. Sample Perishability Limit

Samples must be received at the Laboratory and analyzed within 24 hours of sample collection. Samples aged more than 24 hours from collection or subjected to unsuitable handling conditions will not be analyzed. The date and time of sample collection must be specified for each sample.

5.2 LABORATORY METHODS

This section describes the reference methods used for the examination of recreational water. Only a general outline of each method is provided below. A detailed description of the analytical procedures employed can be found in the "Handbook of Analytical Methods for Environmental Samples - Microbiological Methods", Ministry of the Environment (MOH, 1984). Alternate methods may be used only if they provide equivalent results to the reference method. Equivalency is determined using the ASTM Performance Characteristics, according to Section D-3870-79.

5.2.1. Escherichia coli

Dufour in his epidemiologic study used the m-TEC-urease method; however, preliminary studies using sewage effluents indicate that the sensitivity and accuracy of the m-TEC-urease and m-TEC-Ig methods are equivalent in the recovery and enumeration of *E. coli*. This finding was supported by MOE, MOH, and Dufour (personal communication). The data further indicate that the m-TEC-Ig method has superior specificity (89%) versus 84% in the m-TEC-urease. An additional consideration is the labour cost involved in the methodologies. m-TEC-urease is a two-step procedure and more labour intensive than the one-step m-TEC-Ig method. It is recommended that performance characteristics be established according to ASTM, Section D-3870-79.

Membrane filter analysis: M-TEC-Ig agar method (Reference Method).

Water sample aliquots are filtered through 0.45 um pore size membrane filters. Membrane filters are placed on M-TEC-Ig agar medium and incubated at 44.5° C for 23 hours using a method which provides a delayed rise in temperature. Following incubation, all yellow or predominantly yellow (greenish-yellow or brownish-yellow) colonies with a yellow or colourless margin, 0.5 mm in diameter or larger, are counted using 10x magnification as *E. coli* colonies. Colonies with a green, turquoise or blue halo appearing in the membrane filter, should not be counted.

5.2.2. Pseudomonas aeruginosa

Membrane filter analysis: m-PA agar method (Reference Method).

Water sample aliquots are filtered through 0.45 um pore size membrane filters. Membrane filters are placed on m-PA agar medium and incubated at 41.5° C for 48 hours. Following incubation, all flat, tan to dark brown or black colonies, using 10x magnification, 0.5 mm or larger, are counted as *P. aeruginosa* colonies.

5.3 DATA INTERPRETATION

The medical officer of health should take appropriate action if:

- a. Certain local conditions (e.g., rainfall and associated runoff, epidemics, sewer bypass) occur that may result in a health risk in the bathing area; or,
- b. the microbiological standards are exceeded.

5.3.1 The Quality Control Approach to Recreational Water

The "Quality Control Model" places the health authorities in the role of quality control officer for the product, namely, the bathing water at a beach (Burger and Dufour, in preparation). The consumer is the bather population that uses the beach. The amount of swimming-associated gastrointestinal illness is regulated by posting the beach when the levels of an indicator bacteria in the water exceed the standard.

As in any quality control procedure, the assumption has been made that the quality control officer is familiar with the factors affecting water quality. This familiarity is gained through adequate sanitary surveys of the beach and its environs. A sufficient number of samples should be taken over an appropriate period of time to fully characterize the possible sources of interference and their variability. Once the sanitary survey has established the potential sources of contamination, then a routine microbiological monitoring program should be carried out:

- a) to ensure that the water quality is being maintained, and,
- b) to identify incidences of possible non-compliance.

Table 5 has been generated from the relationship between seasonal bather illness rates and seasonal *E. coli* geometric means (Dufour, 1984) as described in Section 4.1.3. The table shows the seasonal number of swimmers affected by gastrointestinal illness versus the seasonal *E. coli* geometric mean. Once an acceptable seasonal swimming-associated illness rate has been chosen, the appropriate *E. coli* geometric mean can be established as the microbiological standard. The quality control model was developed to assist the quality control officer in determining whether the microbiological data collected throughout the swimming season will comply with this seasonal *E. coli* standard.

The quality control limits provided in Table 6 and illustrated in Figure 2 are based on the quality control model described by ASTM (1951) and modified by Burger and Dufour (in preparation). These control limits were generated with Toronto area microbiological data using:

- a) a standard deviation of 0.7 (e.g., monthly standard deviations on Toronto area data ranged from 0.1 to 1.5), and,
- b) a one-sided 95% upper confidence level as the control limit. (This means that there is a 1 in 20 chance that a sample can exceed the control limit without actually exceeding the established standard).

TABLE 5: SWIMMING ASSOCIATED GASTROINTESTINAL ILLNESS RATES (PER 100,000 SWIMMERS) AND CORRESPONDING SEASONAL GEOMETRIC MEANS FOR E. COLI.

SEASONAL ILLNESS PER 100K SWIMMERS	SEASONAL GEOMETRIC MEAN E. COLI PER 100 ML
50	20
100	23
150	26
200	29
250	33
300	37
350	42
400	47
450	53
500	60
550	68
600	77
650	87
700	99
800	130
900	160
1000	210

TABLE 6: QUALITY CONTROL LIMITS FOR E. COLI

COLUMN		3	4	5	6	7	8	9	10	11	12	13	14	15
1	2	UPPER CONTROL LIMITS FOR GEOMETRIC MEANS												UPPER CONTROL LIMITS FOR SINGLE SAMPLES
SEASONAL ILLNESS PER 100K SWIMMERS	SEASONAL GEOMETRIC MEAN E. COLI PER 100 ML	NUMBER OF SAMPLES												
		5	6	7	8	9	10	11	12	13	14	15	20	
50	20	65	59	54	51	48	46	44	43	41	40	39	36	286
100	23	75	68	62	58	55	53	51	49	48	46	45	41	329
150	26	85	77	71	66	63	60	58	56	54	52	51	47	372
200	29	95	85	79	74	70	67	64	62	60	59	57	52	414
250	33	108	97	90	84	80	76	73	71	69	67	65	69	472
300	37	122	110	101	94	89	85	82	79	77	75	73	67	529
350	42	138	124	115	108	102	97	93	90	87	85	83	76	600
400	47	154	139	128	120	114	109	105	101	98	95	93	85	672
450	53	174	157	145	136	129	123	118	114	111	108	105	96	757
500	60	197	178	164	154	146	139	134	129	125	122	119	109	857
550	68	223	201	186	174	165	158	152	147	142	138	135	123	972
600	77	253	228	210	197	187	179	172	166	161	157	153	140	1100
650	87	286	258	238	223	211	202	194	187	182	177	173	158	1240
700	99	325	293	271	254	240	230	221	213	207	202	197	179	1410
800	130	427	385	355	333	315	301	290	280	272	265	258	236	1860
900	160	526	474	437	410	388	371	357	345	335	326	318	290	2290
1000	210	690	622	574	538	510	487	468	453	439	427	417	381	3000
DEFINITIONS:														
GM <u>E. COLI</u> PER 100 ML = 10 0.001064 ILLNESS plus 1.249 (CALCULATED TO 2 SIG. FIGS.)														
CL ON SINGLE SAMPLE = 10 LOG10 (<u>E. COLI</u>) plus 1.65 STD (CALCULATED TO 3 SIG. FIGS.)														
CL ON GM <u>E. COLI</u> = 10 LOG10 (<u>E. COLI</u>) plus 1.65 STD(SQR(N)) (CALCULATED TO 3 SIG. FIGS.)														
WHERE N equals NUMBER OF SAMPLES AND STD equals STANDARD DEVIATION (0.7)														

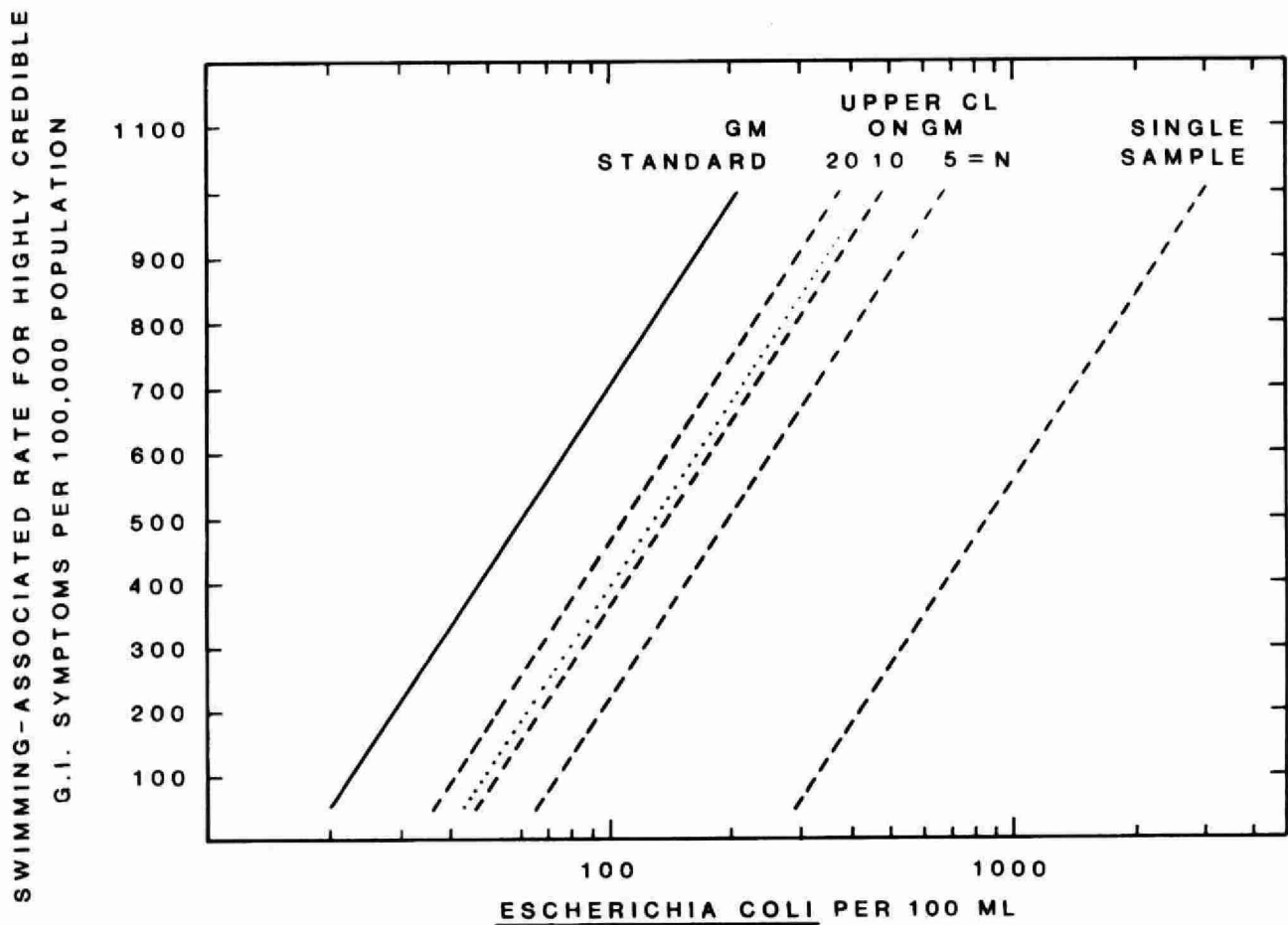


Figure 2: Quality Control Limits for E. coli.

Table 6 provides the following information for the quality control officer:

Columns 1 and 2 provide the seasonal swimming-associated gastrointestinal illness rate per 100,000 swimmers and the associated seasonal geometric mean for *E. coli* given in Table 5.

Columns 3 to 14 provide the corresponding control limits for *E. coli* based on geometric means of the results of 5 to 20 samples. Since the quality control model is independent of time, (provided that all data is subject to the same interferences), the geometric means for data collected on a single day or over a period of time can be compared to the control limits. Similarly, the geometric mean of data from one or many sampling locations representing the same water source can be compared to the control limits.

Column 15 provides the control limits for a single sample. Exceedance of these control limits merely provides a warning that the established standard may be exceeded.

The quality control limits provided in Table 6 may be used as a guide by regulatory agencies in Ontario. These agencies can also generate their own quality control limits using their own data and experience. It is recommended that these agencies consult with the MOE regarding the appropriate statistical procedures.

5.3.2 How to Use the Quality Control Model

The quality control approach provides the health agencies with a procedure for interpreting the microbiological data generated throughout the swimming season, thus allowing decisions to be made regarding possible non-compliance with the predefined water quality standard.

For example:

- a) If a seasonal gastrointestinal illness rate of 700 per 100,000 swimmers has been accepted, the seasonal geometric mean *E. coli* level of 99 per 100 mL would be the standard.
- b) In Table 6, a geometric mean greater than 325 per 100 mL for a set of 5 samples (Column 3) indicates non-compliance with the seasonal standard. Similarly, the geometric mean for 6 samples should not exceed 293 (Column 4) and the geometric mean for 10 samples should not exceed 230 (Column 8) to ensure compliance with the seasonal *E. coli* geometric mean of 99.

- c) On the other hand, a single sample result in excess of 1410 (Column 15) merely provides warning of possible non-compliance. In this situation, immediate additional sampling is warranted. A minimum of 4 additional samples is recommended to allow calculation of a geometric mean for comparison to the control limits provided in Column 3.

It is recommended that the results which have been affected by specific local conditions (e.g., sewage bypass, rainfall) should be excluded from the calculations of geometric means for quality control purposes.

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PATHOGENIC BACTERIA

Salmonella:

The *Salmonella* group are Gram-negative, motile, rod-shaped bacteria that ferment glucose but not lactose. They are the causative agent of Salmonellosis, a highly contagious disease whose symptoms include acute gastroenteritis, enteric fever and septicemia. *Salmonella* are widely distributed and can be found in raw sewage, effluents from packing houses and stormwaters (Reasoner, 1976; Hoadley *et al*, 1974; Geldreich *et al*, 1968; and Claudon *et al*, 1971). Effective sewage treatment and disinfection can greatly reduce the numbers of salmonella in sewage effluents (Yaziz and Lloyd, 1979).

Shigella:

The genus *Shigella* are Gram-negative, non-motile, lactose negative organisms that do not produce hydrogen sulphide. They have been isolated from feces of warm blooded animals and sewage but tend to survive only for a relatively short time in the environment. *Shigella* are the main etiological agents of bacillary dysentery (Lund, 1978). The symptoms of shigellosis typically range from mild transitory diarrhea to vomiting, abdominal pains, fever and profuse bloody feces. Shigellosis can be transmitted through person to person contact, contaminated food and drinking water and although less commonly, through bathing in contaminated waters. A severe outbreak of shigellosis associated with swimming in the highly polluted Mississippi River below Dubuque, Iowa was the first epidemic of shigellosis to be linked with bathing water (Rosenberg, *et al* 1976).

Staphylococcus aureus:

Staphylococcus aureus are Gram-positive, coagulase positive, spherical bacteria occurring in irregular clusters. *S. aureus* is the major pathogen of the genus *Staphylococcus* and is a causative agent of purulent infections, such as boils and infected cuts and scratches (Evans, 1977). The presence of *S. aureus* in recreational waters is generally attributed to discharges from the mouth, nose, throat and body surfaces of swimmers. They are not considered to be natural inhabitants of the aqueous environment. Studies by Brown, *et al* (1984) have shown that staphylococci may be 13 times more chlorine resistant than *E. coli*. Other studies suggest *Staphylococcus aureus* are present in relatively low numbers at Canadian beaches (MOE 1978, Seyfried 1973, 1980).

Legionella:

Legionella spp., the bacteria responsible for Legionnaires disease and Pontiac fever, have been isolated from surface waters. Current information suggests that the *Legionella* spp. are not commonly found in Canadian surface waters (Dutka *et al.*, 1983). On the basis of available evidence it can be concluded that *Legionella* spp. do not pose a hazard in recreational waters in Canada.

VIRUSES

Viruses are minute infectious agents (total diameters range from 20-250 nm) that multiply only inside living cells of susceptible host tissue. Viruses that multiply extensively in the human gastrointestinal tract, e.g., enteric viruses, may contaminate recreational waters following their excretion in feces and may present a potential health hazard. These human enteric viruses include enteroviruses, rotaviruses, enteric adenoviruses, enteric coronavirus, and several morphological categories of small, round, 25-35 nm viruses, such as Norwalk agent. There are at least one hundred serotypes of enterovirus. These viruses may produce various diseases and syndromes in humans, ranging from mild diarrhea to acute gastroenteritis, poliomyelitis, hepatitis, or meningitis (Melnick *et al.*, 1978). Members of virus families that are not cultivated routinely in tissue culture may be detected by direct electron microscopic examination of either feces from patients (McLean and Wong, 1984), or samples of recreational waters (Sobsey, 1982). Certain immunoassays, which may or may not involve electron microscopy, may also be used to detect these non-cultivable viruses (Doane *et al.*, 1983).

Enteric viruses may be excreted in human feces in concentrations as high as 10^8 virus particles per gram. Conventional sewage treatment does not entirely eliminate viruses, and they are released into receiving waters through sewage effluents (England *et al.*, 1967).

Several studies in Ontario have confirmed that sewage treatment plant effluents, even after disinfection, contribute viruses to surface waters (Jenkins *et al.*, 1982; Sattar *et al.*, 1978; Subrahmanyam *et al.*, 1977).

Enteric viruses can also enter surface water in runoff from farm and agricultural lands contaminated with animal waste (Melnick *et al*, 1978). In Ontario, viruses have been isolated from a beach site on the Scugog River (Subrahmanyam, 1977), and from two Conservation Areas in Southern Ontario (Jenkins and Cherwinsky, 1982).

Epidemiological evidence for the waterborne transmission of viruses is limited. Cabelli and Dufour have shown that a significant risk of contracting swimming-associated gastrointestinal disease may exist in recreational water meeting the current microbiological criteria in the United States (Cabelli, 1983a; Dufour, 1984). They suspect Norwalk agent or an unidentified "swimmers" virus, to be the agent involved (Cabelli 1981; Cabelli 1983a; Dufour 1984).

It is evident that Norwalk agent plays a major role in waterborne gastroenteritis in the United States. In a recent outbreak caused by Norwalk agent in Michigan, 121 persons became ill after swimming, even though coliform densities were within EPA limits (Baron *et al*, 1982).

No outbreaks of hepatitis A have been substantiated as a result of contact with recreational waters. The suspected water-borne transmission of poliovirus has not been confirmed by epidemiological evidence (IAWPRC Study Group, 1983).

The health hazard posed by the presence of viruses in recreational waters is dependent upon the minimum infectious dose and the susceptibility of the host. No data are available on the minimum infectious dose of rotavirus, Norwalk agent, or hepatitis A virus but there is reason to believe that for certain highly susceptible individuals a single infectious virus particle is sufficient to cause infection.

Available epidemiological evidence does not indicate that widespread outbreaks of viral infections are occurring via recreational water. However, it must be emphasized that available data may not accurately reflect the role of recreational water in virus transmission (IAWPRC, 1983). The long incubation period of some viral diseases, the transient nature of viral pollution of water, and the inherent difficulties involved in the identification of viral infections, are all factors that make it extremely difficult to recognize waterborne viral diseases. The recent epidemiological studies conducted by Cabelli and Dufour and by Seyfried *et al* in Ontario, indicate that the role of recreational water in the transmission of viral disease may be severely underestimated (Cabelli, 1981; Cabelli, 1983a; Dufour, 1984; Health & Welfare Canada, 1981).

To date, no relationship has been found between the numbers of viruses and indicator bacteria in recreational water (IAWPRC, 1983). It is likely that no adequate bacterial indicator will ever be found that correlates accurately with virus levels (Berg, 1978).

OTHER PATHOGENIC ORGANISMS

Candida albicans:

The yeast *Candida albicans* is regarded as a normal component of the body flora of humans and some animals (Winner and Hurley, 1964). Under certain conditions it can become pathogenic and cause infections of the mouth, vagina, skin and eyes (Gentle and LaTouch, 1969; Beneke and Rogers, 1970, Emmons *et al*, 1977). *Candida albicans* has been isolated from raw sewage and unchlorinated sewage effluents but is rare in properly treated sewage (Ahearn, 1973; Buch and Bubucus, 1978; Sherry *et al*, 1979a). It is known to survive for several weeks in the aquatic environment but reproduction outside a host is regarded to be unlikely. However, Brison (1975) reported an increased incidence of vaginal infections among women frequenting marine beaches contaminated with yeast. Studies of Canadian beaches reported low number of this organism, with elevated levels coinciding with high fecal coliform counts (Sherry *et al* 1979b). The association between candidiasis and recreational waters has not been conclusively established.

Naegleria fowleri:

Naegleria fowleri is an amoeba that has been identified as the main causative agent of primary amoebic meningoencephalitis (PAME) which is usually fatal in humans. Fatal cases of PAME were initially recognized in South Australia (Fowler and Carter, 1965) and in Florida (Butt 1966). *Naegleria fowleri* can exist in two forms in the environment: as an inactive, non-pathogenic cyst in soil or water and as a pathogenic, flagellated, "swimming" amoeba.

The non-infectious form of *Naegleria fowleri* is ubiquitous in the soils of most countries. There are several mechanisms, including runoff by which they enter bodies of water. The cysts may develop into the infectious "swimming" form in warm, organically polluted waters. (Carter 1978).

Two recent studies in the United States investigated the distribution of *Naegleria fowleri* in recreational waters. Wellings (1979), found *Naegleria fowleri* to be widely distributed in lakes in Florida, where the ambient water temperature is naturally high. Duma (1980), showed that the occurrence of *Naegleria fowleri* in lakes in

Virginia increased significantly when water temperatures equalled or exceeded, 30°C. A study in Ontario found *Naegleria* spp. to be widely distributed in sewage effluents and in the Great Lakes. In the same study, *Naegleria fowleri* were isolated from two beach sites one on Lake Ontario and one on Lake Erie (Health and Welfare Canada, 1981). None of these studies differentiated between the cyst and the flagellated forms.

Naegleria fowleri would not be expected to present a health hazard in most Ontario waters since the ambient water temperatures are too low to promote the necessary development of this organism. No cases of PAME have ever been reported in Ontario (MOH data).

Schistosomatidae:

Schistosomiasis or "swimmers itch" is caused by the cercariae of certain schistosomatidae which parasitize birds and rodents. The most important dermatitis producing cercariae are parasites of waterfowl. The eggs are excreted in the bird feces and hatch into flagellated miracidia in the aquatic environment. The flagellated miracidia swim around seeking a specific snail host. Upon accidental contact with man, the miracidia may penetrate the human skin. Since man is an unsuitable host, these parasites die just beneath the epidermis, leaving a protein residue. Subsequent exposure to these cercariae can stimulate an allergic response with progressive severity following each exposure. Schistosomiasis is a problem in Ontario lakes which support populations of the appropriate snail host (Health & Welfare Canada, 1983).

Additional information on Schistosomiasis and its control is available through the Hazardous Contaminants and Standards Branch of the Ministry of the Environment. (Facts Sheet Number 10-02-01).

CANDIDATE INDICATORS FOR FUTURE STANDARDS

ENTEROCOCCUSDescription

Enterococcus group are large, ovoid, catalase negative, Gram-positive bacteria which grow at 10° and 45°C in brain heart infusion broth. Enterococci are a part of the Lancefield's Classification Group D Streptococci. The enterococcus target organism is defined by the typical colonies described by Levin, *et al* (1975). The two principal members encountered are *Streptococcus faecium* and *Streptococcus faecalis*.

Occurrence and Source

Enterococci (*S. faecalis* and *S. faecium*) are found in the feces of warm blooded animals. There is little information on the levels of enterococci in Ontario's recreational waters.

In the past, enterococci levels were not routinely enumerated in Ontario. Instead, the fecal streptococcus test was done. Because it was found that streptococci from non-fecal sources were quite ubiquitous and interfered with the accuracy of the fecal streptococcus test, the fecal streptococcus objective of 20/100 mL of water for recreational use was abandoned in 1978.

The specified enterococcal test described by Levin is much more specific for *S. faecalis* and *S. faecium* which are typically associated with feces.

Relationship to Human Health

In studies previously described in section 4.1.3., Cabelli (1983a) and Dufour (1984) found a significant relationship between the incidence of gastrointestinal disease in swimmers and densities of enterococcus in recreational waters. The linear relationship between enterococcus densities and incidence of Highly Credible Gastrointestinal Symptoms in bathers at freshwater beaches is illustrated in Figure 1: In freshwater environments, correlation coefficients were similar for both enterococcus and *E. coli* at 0.744 and 0.804, respectively. In marine waters, the enterococcus exhibited a stronger correlation to bather illness than did *E. coli*.

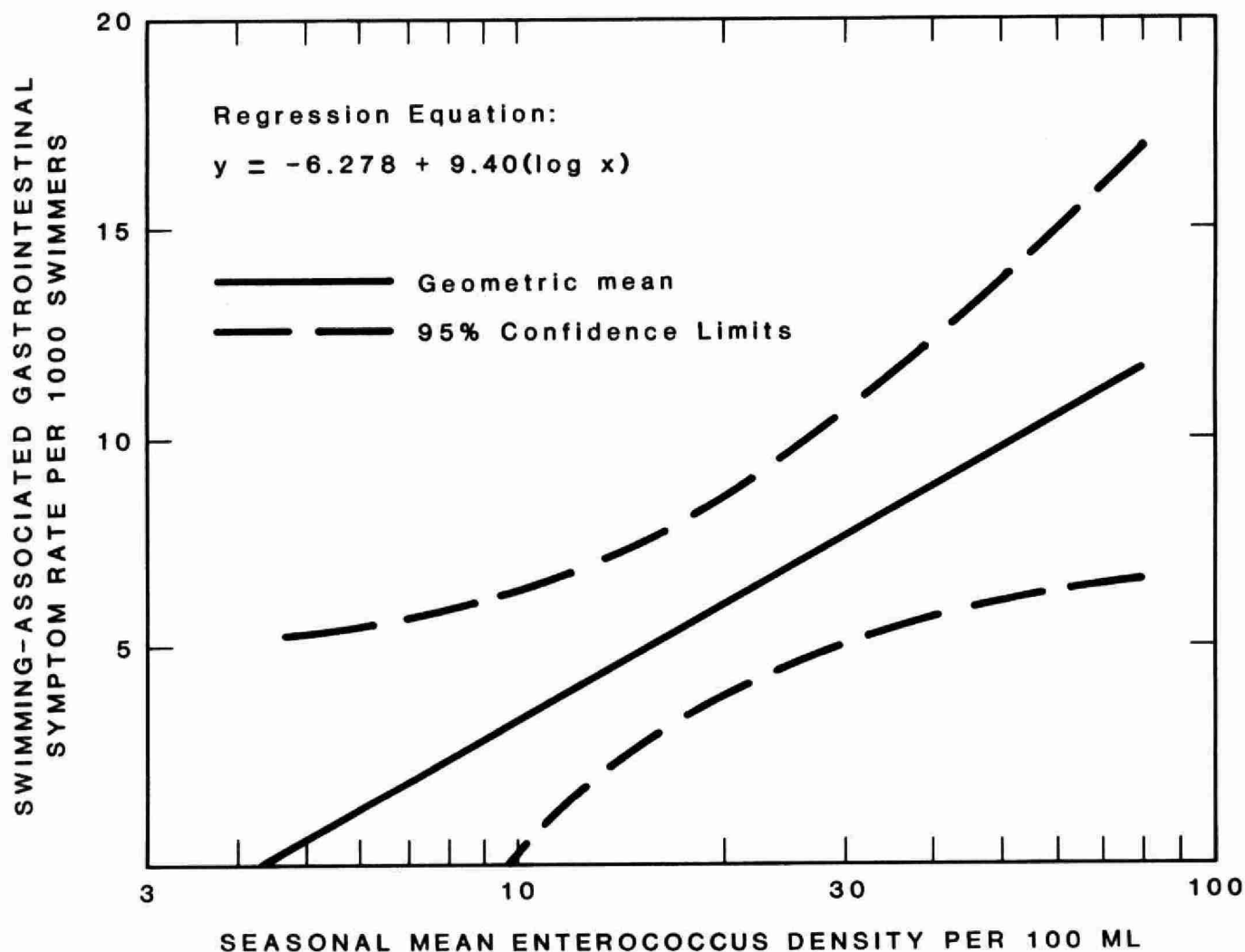


Figure 1: Relationship between swimming associated gastrointestinal disease and Enterococcus (Seasonal G.M. densities, 95% confidence limits), in recreational waters (from Dufour 1984).

TOTAL STAPHYLOCOCCUS

Description

Organisms of the genus *Staphylococcus* are Gram-positive, catalase positive, ferment glucose, produce acid anerobically, and exist as irregular, small, ovoid cells.

Membrane filter analyses are done using Vogel-Johnson agar, supplemented with 0.5% filter sterilized sodium pyruvate (Reference method).

Water sample aliquots are filtered through 0.45 um pore size membrane filters. Membrane filters are placed on supplemented Vogel-Johnson agar and incubated at 35°C for 24 to 48 hours. Following incubation, all black, shiny, and round colonies are counted as total staphylococci.

Sources and Occurrence

Staphylococci occur and persist in lake and river waters but are not believed to flourish under these conditions since Staphylococci prefer temperatures above 20° C and have fairly complex nutrient requirements. The presence of Staphylococci in recreational waters has been linked to bather densities. Staphylococci can be introduced by nose and throat discharges as well as being washed off the skin surfaces of bathers (Oriz, 1977).

Relationship to Public Health

Robinson and Mood (1966) observed that fecal organisms comprised a minority of the bacteria shed by swimmers, in contrast to the staphylococci which were shed in high numbers. Brown (1983) recommended the use of total staphylococci as an indicator of water quality.

Limited data are available on the occurrence of Staphylococci in Ontario waters. Seyfried and Brown (1983) reported that the total staphylococci seasonal mean at nine Ontario beaches ranged from 3 to 236 per 100 ML of water. The overall geometric mean was reported as 150 per 100 mL. Brown (1983) found a positive relationship between local morbidity in the bather population and concentrations of Staphylococci. Brown concluded that 5.5% of swimmers could become ill at a level of 10 total staphylococci per 100 mL of water. At 100 staphylococci per 100 mL, about 10% would be affected and at 1000 staphylococci per 100 mL the total morbidity rate could reach 18% of the swimmers.

RECOMMENDATIONS FOR ADDITIONAL RESEARCH

1. Epidemiological studies;
 - a) to further refine the relationship between bather illness and *E. coli*, *P. aeruginosa*, enterococcus and total staphylococcus indicator organism densities in Canadian waters.
 - b) to determine and quantify other factors that influence the bather illness and indicator organism density relationship, e.g. variations in sampling locations, different sample types (sediment) and meteorological factors.
 - c) Epidemiological studies to assess the public health significance of viruses and other pathogens in recreational waters.
2. Development of a suitable laboratory protocol for enterococcus.
3. Development of improved methods for the recovery and enumeration of viruses from environmental samples (e.g. Norwalk agent and Hepatitis A virus).



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